CLAIMS

What is claimed is:

1. A method for selecting tester proteins capable of binding to a target peptide or protein, the method comprising:

expressing a library of tester proteins in yeast cells, each tester protein being a fusion protein comprised of a first polypeptide subunit whose sequence varies within the library, a second polypeptide subunit whose sequence varies within the library independently of the first polypeptide, and a linker peptide which links the first and second polypeptide subunits;

expressing a target fusion protein in the yeast cells expressing the tester proteins, the target fusion protein comprising a target peptide or protein; and

selecting those yeast cells in which a reporter gene is expressed, the expression of the reporter gene being activated by binding of the tester fusion to the target fusion protein.

2. The method of claim 1, wherein

expressing the library of tester fusion proteins includes

transforming a library of tester expression vectors into the yeast cells which contain a reporter construct comprising the reporter gene whose expression is under transcriptional control of a transcription activator comprising an activation domain and a DNA binding domain, each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator,

a first nucleotide sequence encoding the first polypeptide subunit,

a second nucleotide sequence encoding the second polypeptide subunit, and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence.

3. The method of claim 2, wherein expressing a target fusion protein includes

transforming a target expression vector into the yeast cells simultaneously or sequentially with the library of tester expression vectors, the target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors; and

a target sequence encoding the target protein or peptide; and

expressing the target fusion protein from the target expression vector.

4. The method of claim 1, wherein the steps of expressing the library of tester fusion proteins and expressing the target fusion protein include causing mating between first and second populations of haploid yeast cells of opposite mating types,

wherein

the first population of haploid yeast cells comprises

a library of tester expression vectors for the library of tester fusion proteins, each tester expression vector comprising

a first transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator,

a first nucleotide sequence encoding the first polypeptide subunit,

a second nucleotide sequence encoding the second

polypeptide subunit, and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence;

the second population of haploid yeast cells comprises a target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding the target protein or peptide; and

either the first or second population of haploid yeast cells comprises a reporter construct comprising the reporter gene whose expression is under transcriptional control of the transcription activator.

5. The method of claim 4, wherein the haploid yeast cells of opposite mating types are $\underline{\alpha}$ and $\underline{a}$ type strains of yeast.

6. The method of claim 5, wherein the mating between the first and second populations of haploid yeast cells of $\underline{\alpha}$ and $\underline{a}$ type strains is in a rich nutritional culture medium.

7. The method of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least $1x10^6$.

8. The method of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least $1x10^{10}$.

9. The method of claim 1, wherein the diversity of the fusion proteins encoded by the library of yeast expression vectors is at least $1x10^{12}$.

17. The method of claim 1, wherein the first and second polypeptide subunits of the fusion protein in the library of tester proteins comprises an antibody heavy-chain variable region and an antibody light-chain variable region respectively.

18. The method of claim 1, further comprising:
isolating the tester expression vector from the selected clones; and
mutagenizing the first and second nucleotide sequences in the isolated tester expression vectors to form a library of mutagenized expression vectors.

19. The method of claim 18, wherein the mutagenesis is selected from the group consisting of error-prone PCR mutagenesis, site-directed mutagenesis, DNA shuffling and combinations thereof.

20. The method of claim 1, wherein the target fusion protein comprises an antigen associated with a disease state.

21. The method of claim 1, wherein the target fusion protein comprises a tumor-surface antigen.

22. The method of claim 1, wherein the target fusion protein comprises a human growth factor receptor.

23. The method of claim 22, wherein the human growth factor is selected from the group consisting of epidermal growth factors, transferrin, insulin-like growth factor, transforming growth factors, interleukin-1, and interleukin-2.

24. The method of claim 1, wherein the protein encoded by the reporter

gene is selected from the group consisting of β-galactosidase, α-galactosidase, luciferase, β-glucuronidase, chloramphenicol acetyl transferase, secreted embryonic alkaline phosphatase, green fluorescent protein, enhanced blue fluorescent protein, enhanced yellow fluorescent protein, and enhanced cyan fluorescent protein.

25. A method for selecting tester proteins capable of binding to a target peptide or protein, the method comprising:

expressing a library of tester proteins in yeast cells, each tester protein being a fusion protein comprised of a first polypeptide subunit whose sequence varies within the library, a second polypeptide subunit whose sequence varies within the library independently of the first polypeptide, and a linker peptide which links the first and second polypeptide subunits;

expressing a plurality of target fusion proteins in the yeast cells expressing the tester proteins, each of the target fusion protein comprising a target peptide or protein; and

selecting those yeast cells in which a reporter gene is expressed, the expression of the reporter gene being activated by binding of the tester fusion to the target fusion protein.

26. The method of claim 25, wherein the steps of expressing the library of tester fusion proteins and expressing the plurality of the target fusion proteins includes causing mating between first and second populations of haploid yeast cells of opposite mating types,

wherein

the first population of haploid yeast cells comprises

a library of tester expression vectors for the library of tester fusion proteins, each tester expression vector comprising

a first transcription sequence encoding either the

10. The method of claim 1, wherein the diversities of the first and second polypeptide subunits are each independently derived from libraries of precursor sequences that are not specifically designed for the target peptide or protein.

11. The method of claim 1, wherein the diversities of the first and second polypeptide subunits are not derived from one or more proteins that are known to bind to the target peptide or protein.

12. The method of claim 1, wherein the diversities of the first and second polypeptide subunits are not generated by mutagenizing one or more proteins that are known to bind to the target peptide or protein.

13. The method of claim 1, wherein the first nucleotide sequence is 5' relative to the second nucleotide sequence.

14. The method of claim 16, wherein the first nucleotide sequence in the library of expression vectors comprises a coding sequence of an antibody heavy-chain variable region, and the second nucleotide sequence comprises a coding sequence of an antibody light-chain variable region.

15. The method of claim 1, wherein the linker peptides expressed by the library of expression vectors provide a substantially conserved conformation between the first and second polypeptide subunits across the library of fusion proteins expressed by the library of expression vectors.

16. The method of claim 1, wherein the conformation of the fusion protein having the first and second polypeptide subunits linked by the linker peptide mimics a conformation of a single chain antibody.

activation domain or the DNA binding domain of the transcription activator,

a first nucleotide sequence encoding the first polypeptide subunit,

a second nucleotide sequence encoding the second polypeptide subunit, and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence;

the second population of haploid yeast cells comprises a plurality of target expression vectors, each of the target expression vector comprising

a second transcription sequence encoding either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding the target protein or peptide; and

either the first or second population of haploid yeast cells comprises a reporter construct comprising the reporter gene whose expression is under transcriptional control of the transcription activator.

27. The method of claim 25, wherein members of the library of tester expression vectors are arrayed as individual yeast clones in one or more multiple-well plates.

28. The method of claim 25, wherein members of the library of target expression vectors are arrayed as individual yeast clones in one or more multiple-well plates.

29. The method of claim 25, wherein the mating is based on clonal mating in which each yeast clone containing a members of the tester expression vectors is mated individually with each of the members of the library of target expression vector.

30. The method of claim 25, wherein the plurality of target expression vectors are a library of expression vectors containing a collection of human EST clones or a collection of domain structures.

31. A kit, comprising:

a first and second populations of haploid yeast cells of opposite mating types,

the first population of haploid yeast cells comprising a library of tester expression vectors for the library of tester fusion proteins, each of the tester expression vector comprising a first transcription sequence encoding

either an activation domain or a DNA binding domain of a transcription activator,

a first nucleotide sequence encoding a first polypeptide subunit,

a second nucleotide sequence encoding a second polypeptide subunit, and

a linker sequence encoding a linker peptide that links the first nucleotide sequence and the second nucleotide sequence;

the second population of haploid yeast cells comprises a target expression vector, the target expression vector encodes

either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors, and

a target sequence encoding the target protein or peptide;

wherein either the first or second population of haploid yeast cells comprising a reporter construct comprises a reporter gene whose expression is under transcriptional control of the transcription activator.

32. The kit of claim 31, wherein the second population of haploid yeast

cells comprises a plurality of target expression vectors, each of the target expression vectors encoding

either the activation domain or the DNA binding domain of the transcription activator which is not expressed by the library of tester expression vectors; and

a target sequence encoding the target protein or peptide.

33. The kit of claim 31, wherein the haploid yeast cells of opposite mating types are α and a type strains of yeast.

34. The kit of claim 31, wherein the first polypeptide subunit comprises an antibody heavy-chain variable region, and the second polypeptide subunit comprises an antibody light-chain variable region.

35. The method of claim 31, wherein the protein encoded by the reporter gene is selected from the group consisting of β-galactosidase, α-galactosidase, luciferase, β-glucuronidase, chloramphenicol acetyl transferase, secreted embryonic alkaline phosphatase, green fluorescent protein, enhanced blue fluorescent protein, enhanced yellow fluorescent protein, and enhanced cyan fluorescent protein.